



# SONY Plate Sorting SOP-test



Plate Sort Settings **Plate Adjustment**

Index Sort  
Please check when you want to add index sorting information.  
Add index sort information

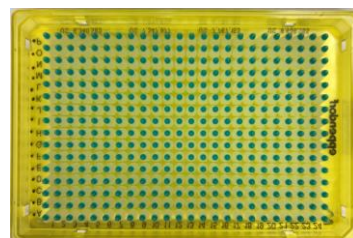
Sort Layout Settings  
☒ Columns to Row (A1 → B1..J) ☐ Row to Column (A1 → A2..J)

Sorting Target Wells  
A 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24  
B  
C  
D  
E  
F  
G  
H  
I  
J

Sort ID: Sort ID 2  
Sort Gate:   
Color:   
Sort Mode: Single Cell  
Stop Count: 1  
Timeout: 0 (Seconds)

Sort ID List:

Sort ID	Sort Gate	Color	Sort Mode	Cell Size	Stop Count	Timeout
Sort ID 1	A		Single Cell	Regular Cell	1	0



## Workflow Overview

SONY SH800 has reliable single cell deposition allowing for reliable 96 & 384 well plate sorting.



## Purpose


Instructions on how to set up successful 384 well plate sorting on the SONY SH800.

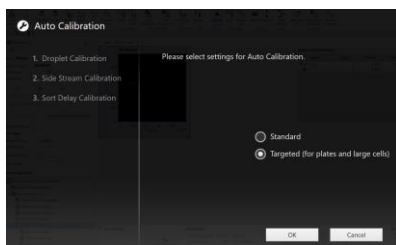
## Supplies Needed

- SONY Automatic Setup Beads.
- TMB ELISA Substrate Solution, Fisher Scientific, Cat # 50-112-9758 (Ready to use, kept at 4°C, protected from light)
- Horseradish Peroxidase (HRP) 10 mg, Fisher Scientific, Cat # PI31490 (make 5 mg/ml stock solution, kept at -20°C)
- Flow-Check Fluorospheres, Beckman Coulter, Cat # 6605359
- 1x Phosphate buffered saline (PBS)
- Multichannel pipette (Volume range is experiment specific)
- Disposable Reagent Reservoir
- Empty 384 well plate (same type of plate as real experiment)
- Adhesive sealing film
- Centrifuge with fixed or swingout rotor for plates
- Accudrop beads

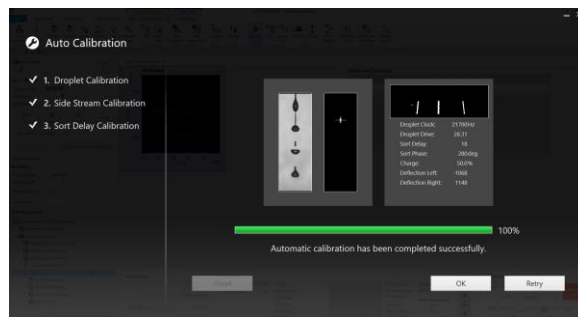


## 1. Startup SONY & QC

- 1 Fill the sheath tank and the sterile water bottle and empty waste tank if needed.
  - 2 Turn on BSC and turn on the air compressor if not using house air. Make sure the air supply to the instrument itself is turned on. 
  - 3 Power on the SH800 and the computer.
  - 4 Log into Windows and launch the SH800 software once the instrument is in Standby mode.
  - 5 Choose 100 µm nozzle chip or 130 µm nozzle chip based on the cells size or cell fragility. Scan the QR code.
  - 6 Clean the deflection plates and sorting chamber with KimWipes moistened by 70% ethanol and dry them afterwards.
  - 7 Follow the software prompts for Automatic Setup using SONY Automatic Setup Beads.
- Note:** See ► **SONY Startup, QC & Shutdown Procedure**
- 8 Choose **Targeted** mode in the Auto Calibration step.




Replace with a new picture showing Targeted chose-Done

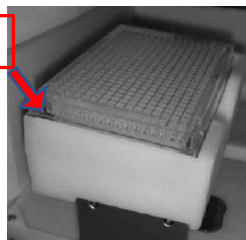


- 9 When the calibration finishes, click **OK**.

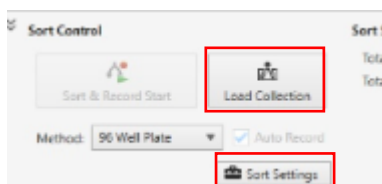
Replace with a new picture-Done

## 2. Experiment setup & plate alignment

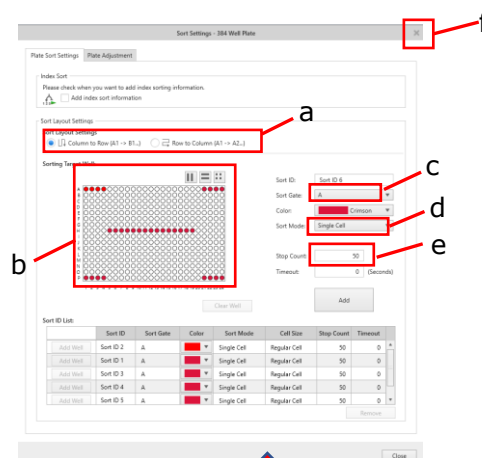
- 1 Create **New Experiment** under *Experiment* Tab and rename it.
- Note:** See ► **SONY Experiment Setup Procedure**.
- 2 Load a tube of Accudrop beads onto the SONY and start acquiring. Set a FSC/SSC gate A around the population of beads.
  - 3 Install the splash guard, as shown in the picture. 
  - 4 Attach the Plate Support Arm to the collection stage.
  - 5 Take the 384 well plate holder out of the fridge and place it on the support arm.
  - 6 Place the empty 384 well plate sealed with a clean adhesive sealing film on the holder. Ensure A1 well is on the front left corner.
  - 7 Click **Load Collection**.
  - 8 Click **Sort Setting** to start plate alignment.



Adapt from SONY Cell Sorter Operation's Guide



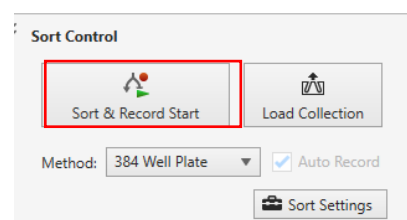
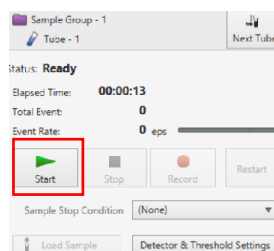
- 9 Under **Plate Sort Settings** tab
  - a) Choose the sorting order:
    - Column to Row
    - Row to Column.
  - b) For alignment step, add some wells on the 4 corners and center for sort testing, for example, A1-4, A21-24, P1-4, P21-24, H5-20.
  - c) Select the gate A to be sorted into the wells.
  - d) Choose **Single Cell** Sort Mode.
  - e) Type the number of events to be sorted in **Stop Count**, such as 50 for testing.
  - f) Exit **Sort Settings** by click the **X** on the up right corner.



Replace it with new picture showing X f-Done

- 10 **Start** acquiring the beads and in the Sort Control pane, click **Sort Start**.

- 11 When sorting is done, take out the plate and examine if the position of each test sort puddle is in the center of the well.



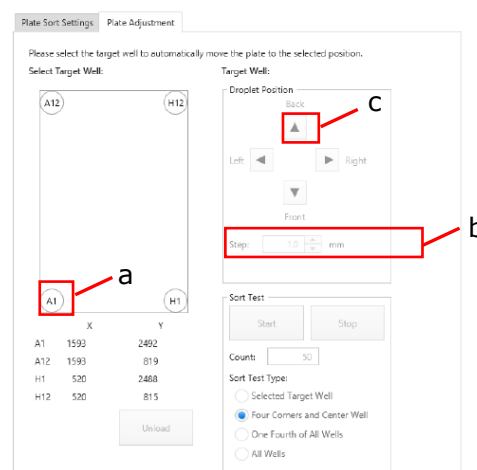
Replace with a new picture-Done

- 12 If any adjustment is needed, go to **Sort Settings** and click **Plate Adjustment** tab.

- a) Choose the position which you want to adjust (A1, A12, H1, or H12).
- b) Set how many millimeters you want to move each step increment.
- c) Click the Droplet Position arrow buttons to move the position of droplet deposition. Each click moves the droplet deposition one step increment.

**Note:** The arrow points to the direction that you want to move the droplets.

- 13 After all necessary adjustments are done, exit **Sort Settings** by clicking the **X** on the up right corner.



- 14 Replace the empty 384 well plate back on the holder. Ensure the A1 well is on the front left corner. Clean and dry the cover film with KimWipes.
- 15 Re-do 50 drops calibration test sort on the cover film. Make more adjustments and repeat the tests as many times as needed.

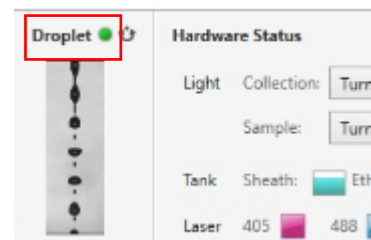
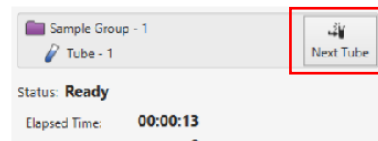
### 3. HRP method verification

HRP method is highly recommended before 384 well plate sorting to verify the single droplet deposition into the bottom of those wells. It is important to use the same volume TMB substrate solution as the volume of buffer or medium in each well.

**Note:** See ►**HRP Method SOP**.

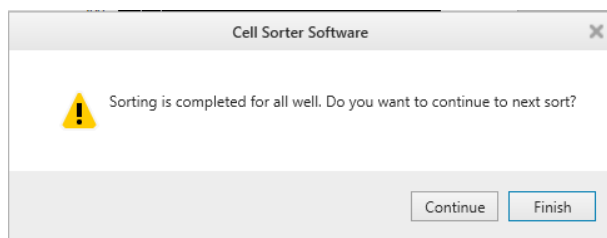
## 4. Plate sorting

- 1 Create **Next Tube** for sample. Load the sample and acquire some cells from the sample and then stop. Create gating strategy.
- 2 Go to **Plate Sort Settings** tab. Select wells and assign the gate to be sorted into those cells and type the number of cell to be sorted each well in **Stop Count**, such as 1 cell/well. Leave the Timeout value set at 0 seconds (disabled).
- 3 Exit **Sort Settings** by click the **X** on the up right corner.
- 4 Place the 384 well plate with collection medium or buffer on the holder. Ensure A1 well is on the front left corner. Open the lid and leave the lid on a clean paper towel outside the sorting chamber.
- 5 Click **Resume** to get the sample running again. Click **Sort Start** in the Sort Control pane.



**Note:** Confirm the sort calibration is valid before sort start (solid green dot in upper right corner of Sort Control pane).

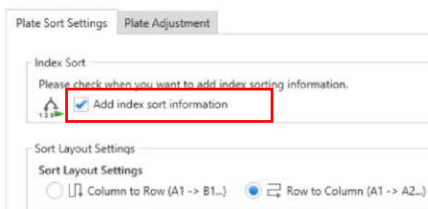
- 6 When sorting is done, a message pops up. Choose **Continue** if multiple plates will be sorted with the exactly same layout. When all plates for the same sample are done, choose **Finish**.



## 5. Index sorting (optional)

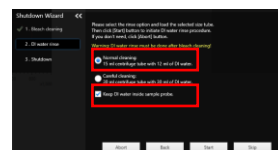
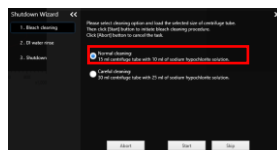
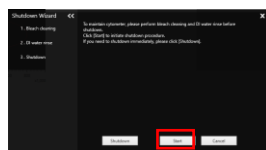
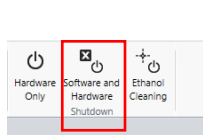
Index sorting is available on the SONY SH800. On the **Plate Sort Setting** tab, activate Index sort by checking the box **Add index sort information**.

**Note:** This must be selected before the plate is sorted.



## 6. Shut down the system

- 1 Export the experiment template and FCS files after the experiment is done.  
**Note:** See ► **SONY Experiment Export Procedure**
- 2 In the Cytometer Tab, click on **Hardware and Software Shutdown** button and follow the prompts.  
**Note:** See ► **SONY Startup, QC & Shutdown Procedure**



- 3 Shutdown the computer.
- 4 Turn off the air compressor or house air supply.

Insert a picture showing turn off the air compressor/ valve-Done

